Clin Exp Immunol 2004; **138**:151–157 doi:10.1111/j.1365-2249.2004.02584.x

Interleukin-8 is involved in cervical dilatation but not in prelabour cervical ripening

Y. SAKAMOTO*, P. MORAN*, R. F. SEARLE†, J. N. BULMER‡ & S. C. ROBSON* **Schools of Surgical and Reproductive Sciences, †Medical Education Development and ‡Clinical and Laboratory Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, UK*

(Accepted for publication 29 June 2004)

SUMMARY

Our aim was to determine the amount and source of interleukin (IL)-8 and to study IL-8 receptor expression in the human cervix during pregnancy and after labour. Cervical biopsies were obtained from six non-pregnant women, eight women undergoing pregnancy termination, 17 women undergoing elective caesarean section and 11 women after vaginal delivery. IL-8 levels were compared in women with and without a ripe cervix, as determined by cervical Bishop score and cervicovaginal fetal fibronectin levels. Levels of IL-8 and IL-1β, a regulator of IL-8 expression, were determined by enzyme-linked immunosorbent assay (ELISA). IL-8, IL-1β and IL-8 receptor proteins were localized by immunohistochemistry. Compared with late pregnancy, IL-8 levels increased after labour and vaginal delivery ($P < 0{\cdot}01$) but there was no correlation with cervical ripening. IL-8 was localized to stromal cells, macrophages and granulocytes. There were no significant differences in IL-1β levels between groups. IL-8 receptors were expressed primarily on granulocytes and macrophages after vaginal delivery. We conclude that IL-8 is involved in cervical dilatation but not in cervical ripening.

Keywords cervix IL-8 IL-8RA(CXCR-1) IL-8RB(CXCR-2) leucocytes pregnancy

INTRODUCTION

For most of pregnancy the uterine cervix remains rigid and closed but prior to labour it undergoes softening and effacement (ripening). Absence of normal ripening at term is associated with prolonged labour and post-term pregnancy, whereas premature ripening occurs as part of the preterm delivery syndrome [1]. Ripening is associated with collagen remodelling and alterations in proteoglycan and water content [2,3]. Collagenolysis is thought to follow infiltration of the cervix by granulocytes with the subsequent release of matrix metalloproteinases (MMP), particularly the collagenase MMP-8 and the gelatinases MMP-2 and MMP-9 [4–6]. Although cervical ripening resembles an inflammatory process, the mechanisms that control granulocyte infiltration during cervical ripening and dilatation are not well defined.

Interleukin (IL)-8 is a proinflammatory cytokine that has strong chemotactic and activating effects on neutrophils leading to the release of collagenase and elastase-containing granules [7]. IL-8 signals through two high affinity receptors: IL-8 receptor type A (IL-8RA) and IL-8 receptor type B (IL-8RB) [8,9] which are differentially expressed on leucocyte subpopulations [10]. IL-8 production has been reported in cervical stromal fibroblasts [11,12], glandular epithelial cells and leucocytes [13,14] and levels of IL-8 in human cervical tissue increase during pregnancy [13]. There is increasing evidence that this cytokine is involved in the cervical changes during labour [2,11] and that IL-1β may regulate the cervical secretion of IL-8 [11,15].

Most previous studies of IL-8 in human cervix have focused on cytokine levels in lower uterine segment myometrium during labour [2,11,16] and there is very little information on the concentration and source of cervical IL-8 during pregnancy and ripening. Furthermore it is unclear which cells in the cervix express IL-8 receptors. The aim of the present study was therefore to determine the amount and source of IL-8 and to study IL-8 receptor expression in the human cervix during pregnancy and after labour in order to shed light on the factors controlling granulocyte influx into the cervix. To determine the role of IL-8 in cervical ripening, levels were compared in women with and without a ripe cervix, as determined by cervical Bishop score and cervicovaginal fetal fibronectin levels. Because IL-1β has been shown to influence IL-8, the levels and source of this cytokine was also determined.

MATERIALS AND METHODS

Subjects

Forty-two healthy women were recruited from the Obstetric and Gynaecology wards at the Royal Victoria Infirmary, Newcastle upon Tyne. Written informed consent was obtained from each subject and the study was approved by the Joint Ethics

Correspondence: Dr Roger F. Searle, Anatomy and Clinical Skills, School of Medical Education Development, The Medical School, Framlington Place, Newcastle upon Tyne NE2 4 HH, UK.

E-mail: r.f.searle @ncl.ac.uk

Committee Newcastle and North Tyneside Health Authority. The non-pregnant group consisted of six regularly menstruating women [median age 42·5 (range 40–43) years, median parity 2 (range 1–4)], undergoing hysterectomy for non-malignant disease. None of the women had cervical disease. The early pregnant group consisted of eight women [median age 25 (range 21–34) years, median parity 1 (range 0–3)] undergoing surgical termination of pregnancy at a median gestational age of 85 (range 56–91) days. None of the women received prostaglandin or a cervical ripening agent prior to surgery. The late-pregnant group consisted of 17 healthy non-labouring pregnant women with no membrane rupture or cervical ripening [median age 32 (range 28–42) years, median parity 1 (range 0–3)] undergoing elective caesarean section at a median gestational age of 274 (range 261–287) days. The indications for caesarean section were previous caesarean section or breech presentation. The vaginal delivery group consisted of 11 women, with a median age of 27 years (range 22–33), a median parity of 0·5 (range 0–2) and a median gestational day of 282·5 days (range 273–288), who had just completed vaginal delivery following spontaneous term labour.

Sample collection

One or two punch biopsies (each 20–25 mg) were taken from the anterior and/or posterior cervical lips using a 6 mm biopsy needle (Stiefel Laboratories, Woburn Green, Bucks, UK). The biopsies from non-pregnant women were taken in the operating theatre immediately after hysterectomy. Cervical biopsies from early pregnant, late pregnant or vaginal delivery groups were taken transvaginally immediately after surgical termination, caesarean section or vaginal delivery. Biopsies were immediately snap-frozen in liquid nitrogen-cooled isopentane and stored at −70°C until assayed.

In women undergoing caesarean section, cervicovaginal fluid for fetal fibronectin measurement, which correlates to cervical ripening, was taken from the posterior fornix using a dacron polyester swab prior to the operation [17]. Cervicovaginal fluid samples were stored at −70°C until required. The cervix was also assessed by digital vaginal examination and scored using Bishop's pelvic score [18] with scores of 0–3 each for cervical dilatation, length and station of the fetal head, and 0–2 for consistency and position. A Bishop score >4 was taken to indicate a ripening cervix, while a score >8 indicated a ripe cervix [18]. For all subjects the same obstetrician (PM) collected the cervicovaginal fluid samples and evaluated the Bishop's pelvic score to standardize clinical evaluations.

Cytokine assay

In order to determine the concentration of IL-8 in cervical epithelium and stroma separately, each biopsy was cut on ice 1 mm from the well-defined epithelial surface. Routine histology confirmed that the 'epithelial' sample contained stratified squamous epithelium with superficial stroma while the 'stromal' sample comprised smooth muscle cells, fibrous tissue, blood vessels and leucocytes with no epithelial elements. Individual epithelial and stromal samples were homogenized in 300 μl RPMI-1640 on ice using Polyton PT3000 for 15 s at 4000 r.p.m. or until complete homogenization was achieved. The homogenate was then centrifuged at 300 ***g*** for 10 min and the supernatant stored at −70°C until assayed. Levels of IL-8 or IL-1β were determined by an enzyme-linked immunosorbent assay (ELISA) using commercially available kits purchased from R&D Systems (Abingdon, Oxon, UK). The limit of detection for IL-8 was 10 pg/ml and for IL-1β was 1 pg/ml. Results were expressed as pg of cytokine per mg total protein as determined by the modified Lowry method using DC protein assay kit (Bio-Rad, CA, USA).

Single immunohistochemical labelling

Immunohistochemistry was performed on 7 μm cryostat sections of intact cervix using an avidin–biotin–peroxidase method (Vectastain Elite mouse kit; Vector Laboratories, Peterborough, UK) [19]. To evaluate the expression of IL-8, IL-1β and IL-8 receptors, sections were incubated with primary monoclonal antibody (MoAb) in TBS (Table 1). Optimal dilutions and incubation times were determined on positive control tissues. With the exception of anti-IL-8RA, bound primary MoAbs were detected by incubation with 0·05% 3,3′-diaminobenzidine-tetrahydrochloride (DAB) (Sigma Chemical Co., Poole, UK) containing 0·03% hydrogen peroxide for 5 min. IL-8RA was detected by incubation with NovaRED (Vector Laboratories). A positive reaction was demonstrated as a brown reaction with DAB or as a red reaction with NovaRED. Negative controls were performed for all samples and sections were incubated with non-immune serum instead of primary MoAb. For positive controls cryostat sections of non-pregnant endometrium were used for IL-8, placental tissue for IL-1β and tonsil tissue for IL-8RA (CXCR-1) and IL-8RB (CXCR-2).

Double immunohistochemical labelling

Double immunohistochemical labelling was used to identify the immunopositive cells in the vaginal delivery group [19]. Briefly, sections were first labelled for IL-8, IL-1β, IL-8RA or IL-8RB using the avidin–biotin–peroxidase method and developed with NovaRED, positive cells staining red. The slides were then

Table 1. Primary monoclonal antibodies used for immunohistochemistry

MoAb and specificity	Blocking serum	Dilution	Incubation time	Incubation temperature	Source	Clone
IL-8	Horse	1/100	overnight	4°C	Biosource[1]	893C4G2
IL-1β	Horse	1/20	overnight	4°C	R&D[2]	8516·331
IL-8RA (CXCR-1)	Horse	1/40	overnight	4°C	R&D	42705·111
IL-8RB (CXCR-2)	Human	1/40	overnight	4°C	R&D	48311·211
CD14; macrophage	Horse	1/10	60 min	Room temperature	Novocastra[3]	M-M-42
CD15; granulocyte	Horse	1/10	60 min	Room temperature	Novacastra	BY87
CD3; T lymphocyte	Horse	1/200	60 min	Room temperature	Novocastra	UCHT1

[1]Biosource International, Camarillo California, USA. [2]R&D Systems, Minneapolis, USA. [3]Novocastra Laboratories Ltd, Newcastle upon Tyne, UK.

incubated with the second primary antibody, anti-CD3, anti-CD14 or anti-CD15 (Table 1), and incubated sequentially with biotinylated antimouse immunoglobulin (30 min) and ABC alkaline phosphatase (30 min) (Vector Laboratories) and developed using alkaline phosphatase substrate III (Vector Blue) (Vector Laboratories), positive cells staining blue. The antibody combinations used were IL-8/CD14, IL-8/CD15, IL-1β/CD14, IL-8RA/CD3, IL-8RA/CD14, IL-8RA/CD15 IL-8RB/CD3, IL-8RB/CD14 and IL-8RB/CD15.

Negative controls included replacement of either the first or second primary antibodies with nonimmune serum. Single- and double-labelled sections were also compared for all samples in order to confirm that there was no spurious double labelling.

Fetal fibronectin assay

Fetal fibronectin was assayed using an enzyme immunoassay kit (ADEZA Biochemistry, Sunnyvale, CA, USA) performed according to the manufacturer's instructions. The limit of detection of the kit is 50 ng/ml and concentrations greater than this were deemed positive and taken to indicate a ripe cervix.

Data and statistical analysis

Statistical analysis was performed with Statview (Berkeley, CA). Data are reported as mean (standard error of the mean). Differences in cytokine levels between groups were compared by analysis of variance (ANOVA). If ANOVA suggested a statistically significant effect, differences between individual groups were compared using Bonferroni/Dunn's *post-hoc* multiple comparison. The correlation between IL-8 concentrations and Bishop score was determined using Spearman's correlation coefficient. $P < 0{\cdot}05$ was considered statistically significant.

RESULTS

Levels of IL-8 and IL-1β *in cervix during pregnancy and after delivery*

Concentrations of IL-8 in stromal samples from the non-pregnant, early pregnant and late pregnant groups were similar; 39·9 (14·6) pg/mg protein, 22·0 (13·6) pg/mg protein and 114·7 (58·6) pg/mg protein, respectively. In contrast stromal IL-8 concentration was increased in the vaginal delivery group (949·2 (305·0) pg/mg protein) relative to non-pregnant and pregnant groups ($P < 0{\cdot}01$, Fig. 1). The levels of IL-8 in the epithelial samples from the non-pregnant, early pregnant and late pregnant groups were similar but after vaginal delivery epithelial IL-8 levels increased ($P < 0{\cdot}01$, Fig. 1). There were no statistically significant differences in IL-8 concentrations between stroma and epithelium.

IL-1β concentrations in stromal and epithelial samples after vaginal delivery increased but showed no significant differences with the non-pregnant and pregnant groups (Fig. 2). There was significant correlation between IL-8 and IL-1β concentrations in stroma and epithelium ($R = 0{\cdot}665$ and $R = 0{\cdot}705$, $P < 0{\cdot}001$, respectively).

Sources of cervical IL-8 and IL-1β *during pregnancy and after delivery*

All negative controls were unstained and positive controls showed the expected immunoreactivity (data not shown).

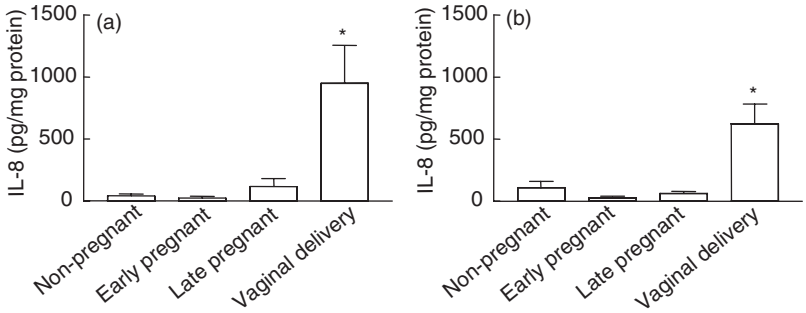

Fig. 1. IL-8 levels (mean ± s.e.m.) in stromal (a) and squamous (b) cervical samples from non-pregnant ($n = 6$), early pregnant ($n = 8$), late pregnant ($n = 17$) and vaginal delivery groups ($n = 11$). *$P < 0{\cdot}01$ *versus* non-pregnant, early pregnant and late pregnant groups.

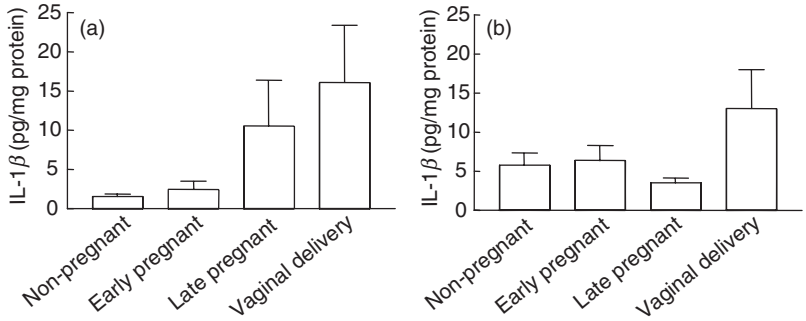

Fig. 2. IL-1β levels in stromal (a) and squamous cervical samples (b) from the non-pregnant ($n = 6$), early pregnant ($n = 8$), late pregnant ($n = 17$) and vaginal delivery groups ($n = 11$).

Squamous epithelial cells were weakly reactive for IL-8 in all samples regardless of the subject group. However, the distribution of IL-8 positive cells in stroma differed dramatically between groups; numerous IL-8 positive cells were seen in cervix from the vaginal delivery group (Fig. 3a) but staining was absent or showed the occasional weak positive cell in samples from the other groups (Fig. 3b).

Staining for IL-1β was similar to that for IL-8. Squamous epithelium was weakly stained in all subject groups. Although there were many IL-1β positive cells in the cervical stroma in samples from the vaginal delivery group (Fig. 3c), staining was absent or occasional weak positive cells, often on blood vessels in the other groups (Fig. 3d).

Double-labelling of the vaginal delivery group samples showed that IL-8 was localized not only to stromal cells but also to many CD15-positive granulocytes (Fig. 3e). A striking finding was the presence of approximately half the CD14-positive macrophages co-labelling with IL-8 (Fig. 3f). A minor population of CD14-positive macrophages outside blood vessels also co-stained for IL-1β (Fig. 3g)

Cervical IL-8 receptor expression during pregnancy and after labour

Immunohistochemical staining for IL-8RA or IL-8RB showed a similar distribution to that for IL-8 (Fig. 3h–k). Extravascular cells that expressed IL-8 receptors were seen only in the cervix from the vaginal delivery group (Fig. 3h,j). In other groups IL-8 receptor expression was confined to cells within blood vessels (Fig. 3i,k). Interestingly, there were many more IL-8RB (Fig. 3h) positive cells than IL-8RA (Fig. 3j) positive cells. Double immunohistochemical labelling revealed that IL-8RB was localized predominantly to CD15-positive granulocytes (Fig. 3l), a population of CD14-positive macrophages (Fig. 3m) and occasional CD3-positive T cells. In contrast IL-8RA was localized to granulocytes (Fig. 3n) and only very occasional macrophages (Fig. 3o,p).

IL-8 levels in women with and without a ripe cervix

In the late pregnant prelabour group five women had a Bishop score >4 but none had a score >8. There was no correlation between Bishop score and IL-8 concentration in either stroma ($n = 17$) or epithelium ($n = 12$). There was no statistically significant difference in IL-8 concentration between women with an unripe (Bishop score 4 or less) and ripening cervix (Bishop score above 4) in either stroma (56·5 (13·1) pg/ml protein *versus* 71·5 (29·1) pg/ml protein) or epithelium (146·0 (82·1) pg/ml protein *versus* 39·4 (7·0) pg/ml protein). Four of the 16 cases tested for fetal fibronectin in the late pregnant group were positive for fetal fibronectin. Three of the four women with a positive fetal fibronectin had a Bishop score >4. Stromal IL-8 concentrations were similar in fetal fibronectin positive and negative cases; 102·0 (64·8) and 115·5 (81·6) pg/mg protein, respectively. Similarly, epithelial IL-8 concentrations were similar in fetal fibronectin positive and negative cases; 59·0 (36·8) and 63·9 (15·2) pg/mg protein, respectively.

DISCUSSION

There is increasing evidence that the process of cervical ripening and dilatation resembles an inflammatory reaction associated with an influx of granulocytes into the cervical stroma. The results of the present study show that levels of the inflammatory cytokine IL-8 increase dramatically in both cervical stroma and squamous epithelium after spontaneous labour and vaginal delivery. Moreover, only the vaginal delivery group was associated with an influx of predominantly granulocytes and macrophages into the cervix. These leucocytes expressed IL-8RA and IL-8RB receptors, inferring that IL-8 may play a crucial role in the recruitment of leucocytes during cervical dilatation. However, we did not demonstrate any increase in IL-8 levels in women with ripening cervices, as evidenced by a Bishop score above 4 or positive fetal fibronectin in cervicovaginal fluid. These results suggest that IL-8 is not involved in cervical ripening.

Previous studies have shown that IL-8 increases in lower uterine segment myometrium during active labour [2,11,20]. However, lower segment myometrium is structurally and functionally different from cervix. Sennstrom *et al*. [13] are the only previous group to study IL-8 levels in human cervical biopsies; they reported an increase in cervical IL-8 protein in term pregnant women with an unripe cervix compared with non-pregnant women, and a further dramatic increase after delivery. The present study confirmed increased IL-8 protein level after delivery in both cervical stroma and epithelium. Recently others have reported that IL-8 increased during labour in amniotic fluid, retroplacental blood and maternal serum [20,21], but this is likely to reflect amniochorial and decidual tissue IL-8 release rather than cervical production.

To determine whether IL-8 was involved in cervical ripening we studied non-labouring women with and without a ripe cervix. We found no correlation between IL-8 levels and Bishop score and no difference in IL-8 levels in women with a ripe cervix, defined as either a Bishop score >4 or positive fetal fibronection. There is a correlation between Bishop score and cervical fetal fibronectin [17] and both predict the time to spontaneous labour [22]. These results suggest that IL-8 levels are independent of early cervical ripening but there are the provisos that the number of women studied was low and that Bishop score is a surrogate for cervical ripening. Importantly, as we did not include women with very favourable cervices, we cannot exclude the possibility that IL-8 increases late in the ripening process. However, this seems unlikely as others have found no change in IL-8 concentrations in lower segment samples prior to and following the onset of labour [2]. This is further supported by data from two cases of emergency caesarean section after the onset of labour, where IL-8 levels and the numbers of infiltrating leucocytes showed similar values to those after vaginal delivery (data not shown), suggesting that the changes take place during labour. An alternative approach would be to sample women in preterm labour who often have very favourable cervices, but there is the concern that the mechanisms involved in cervical ripening and dilatation is likely to differ from that in spontaneous term labour.

Although we could not identify the initial trigger for cervical ripening, potential candidates include mechanical stretch, relaxin, nitric oxide, insulin-like growth factor 1, dithiol redox enzymes, prostaglandins and other cytokines/chemokines (including IL-1α, IL-6, RANTES, MCP-1, PAF) [23–29]. Stjernholm *et al*. [30,31] have also shown that labour is associated with a down-regulation of oestrogen and progesterone receptor levels in the cervix and a switch to a more ERβ influenced state. However, none of the studies implicating these mediators has reported prelabour levels in ripe and unripe cervices. Thus interpretation is confounded by the effects of labour.

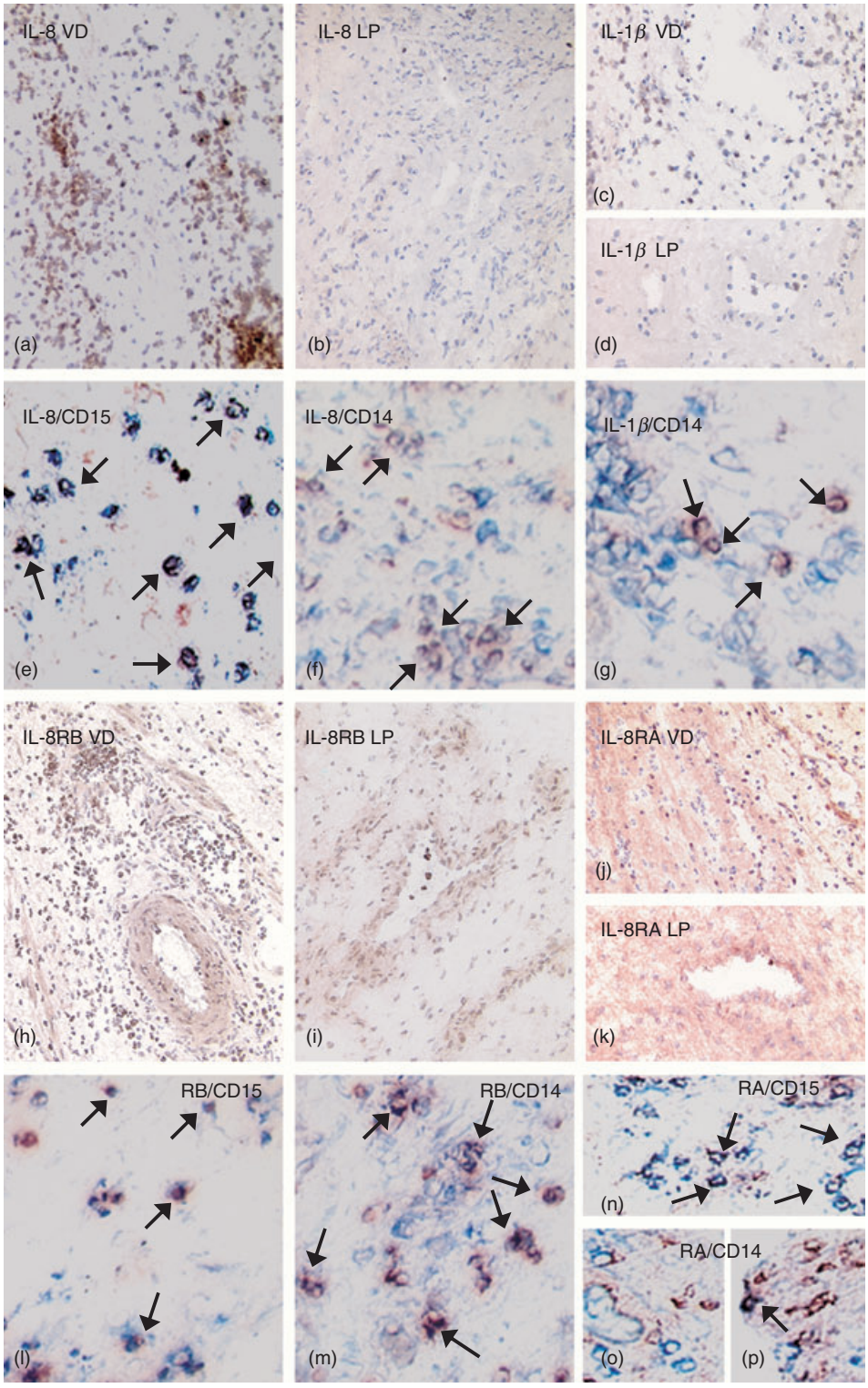

Fig. 3. (a–d) IL-8 (a, b) and IL-1β (c, d) localized in the vaginal delivery (VD; a, c) and late pregnancy (LP; b, d) groups. IL-8$^+$ and IL-1β^+ cells are seen easily in the vaginal delivery group but are rare in the late pregnancy group. (e–g) Vaginal delivery group double-labelled for IL-8/CD15 (e), IL-8/CD14 (f) and IL-1β/CD14 (g). Single-labelled IL-8$^+$ and IL-1β^+ cells are red, CD14$^+$ and CD15$^+$ cells are blue and double-labelled cells appear deep blue/purple (arrowed). (h–k) IL-8RB (h, i) and IL-8RA (j, k) localized in the vaginal delivery (h, j) and late pregnancy (i, k) groups. Numerous IL-8RB$^+$ and IL-8RA$^+$ cells are seen in the vaginal delivery group, but positive cells in other groups are located within blood vessels. (l–p) Vaginal delivery group samples double-labelled for IL-8RB(RB)/CD15 (l), IL-8RB/CD14 (m), IL-8RA (RA)/CD15 (n) and IL-8RA/CD14 (o, p). IL-8RB$^+$ and IL-8RA$^+$ cells are red, CD15$^+$ and CD14$^+$ cells are blue and double-labelled cells are purple (arrowed). Many CD15$^+$ granulocytes express IL-8RA and IL-8RB, but whereas IL-8RB which is expressed by a substantial proportion of CD14$^+$ macrophages, only rare CD14$^+$ macrophages express IL-8RA. Magnification a–d, h–k ×200; e–g, l–p ×400.

In the cervical stroma, expression of IL-8RA and IL-8RB receptors was seen only after vaginal delivery. IL-8RB positive cells (granulocytes, macrophages and T lymphocytes) predominated, although granulocytes also expressed IL-8RA. This pattern of IL-8 receptor expression concurs with the composition of the inflammatory cell infiltrate, predominantly granulocyte and macrophage, identified in the myometrium and cervix during parturition [14,32,33]. These findings suggest that IL-8, produced locally within the cervix, is at least partly responsible for the recruitment of granulocytes and macrophages during active labour. The fact that the time-course of neutrophil invasion and collagenase production closely parallels the increase in IL-8 levels in the cervix [34] strongly supports this.

Although fibroblasts, glandular epithelial cells and leucocytes in the cervix have all been shown to produce IL-8 [11–14], the dramatic up-regulation in IL-8 and the concomitant influx of macrophages and granulocytes with labour and vaginal delivery concurs with reports that infiltrating leucocytes are a major source of proinflammatory cytokines in uterine tissues during labour [14,35]. The present demonstration that these cells also express IL-8 receptors indicates an autocrine/paracrine regulation of IL-8 within the cervix.

Stromal cells and macrophages showed immunostaining for IL-1β after labour and vaginal delivery but we did not find a statistically significant increase in IL-1β concentrations in either cervical stroma or epithelium. However, IL-1β levels were variable and a larger study may demonstrate an increase in late pregnancy and labour. Further, the correlations between IL-8 and IL-1β concentrations in cervical stroma and epithelium are consistent with IL-1β regulating IL-8 production in the cervix [11]. It has been reported recently that IL-1β induces glycosaminoglycan synthesis in cervical fibroblasts *in vitro* via a PGE_2 pathway and up-regulated EP4 receptor mRNA expression [36]. Further studies are clearly needed to clarify the precise role of IL-1β, if any, in the events of cervical ripening and dilatation.

In summary, IL-8 is crucially involved in cervical dilatation but not in prelabour cervical ripening. Up-regulated IL-8 production in the cervix after labour and vaginal delivery is associated with infiltrating macrophages and granulocytes. IL-8 receptor expression on the influx of leucocytes into the cervix suggests that an autocrine/paracrine mechanism enables the increase in IL-8 production in cervical dilatation.

ACKNOWLEDGEMENTS

We thank Mrs Barbara Innes for assistance with immunohistochemistry, the labour and operating ward staff of the Royal Victoria Infirmary for their assistance with sample collection and Dr Simi Ali, University of Newcastle upon Tyne for helpful discussions. Dr Yoshiharu Sakamoto was supported by the Researcher Overseas Programme of Nara Medical University, Japan.

REFERENCES

1 Olah KS, Gee H. The prevention of preterm delivery – can we afford to continue to ignore the cervix? Br J Obstet Gynecol 1992; **99**:278–80.
2 Osmers RGW, Blaser J, Kuhn W, Tschesche H. Interleukin-8 synthesis and the onset of labour. Obstet Gynecol 1995; **86**:223–9.
3 Osmers R, Rath W, Pflanz MA, Kuhn W, Stuhlsatz HW, Szeverenyi M. Glycosaminoglycans in cervical connective tissue during pregnancy and parturition. Obstet Gynecol 1993; **81**:88–92.
4 Junqueira LC, Zugaib M, Montes GS, Toledo OM, Krisztan RM, Shigihara KM. Morphologic and histochemical evidence for the occurrence of collagenolysis and for the role of neutrophilic polymorphonuclear leukocytes during cervical dilation. Am J Obstet Gynecol 1980; **138**:273–81.
5 Osmers R, Rath W, Adelmann-Grill BC *et al.* Origin of cervical collagenase during parturition. Am J Obstet Gynecol 1992; **166**:1455–60.
6 Winkler M, Oberpichler A, Tschesche H, Ruck P, Fischer DC, Rath W. Collagenolysis in the lower uterine segment during parturition at term. Correlations with stage of cervical dilatation and duration of labor. Am. J Obstet Gynecol 1999; **181**:153–8.
7 Baggiolini M, Wakz A, Kunkel SL. Neutrophil activating peptide-1/interleukin-8 a novel cytokine that activates neutrophils. J Clin Invest 1989; **84**:1045–9.
8 Holmes WE, Lee J, Kuang WJ, Rice GC, Wood WI. Structure and functional expression of a human interleukin-8 receptor. Science 1991; **253**:1278–80.
9 Murphy PM, Tiffany HL. Cloning of complementary DNA encoding a functional human interleukin-8 receptor. Science 1991; **253**:1280–3.
10 Chuntharapai A, Lee J, Hebert CA, Kim KJ. Monoclonal antibodies detect different distribution patterns of IL-8 receptor A and IL-8 receptor B on human peripheral blood leukocytes. J Immunol 1994; **153**:5682–8.
11 Winkler M, Fischer DC, Hlubek M, Van de Leur E, Haubeck HD, Rath W. Interleukin-1beta and interleukin-8 concentrations in the lower uterine segment during parturition at term. Obstet Gynecol 1998; **91**:945–9.
12 Winkler M, Rath W, Fisher DC, Van de Leur E, Haubeck HD. Regulation of interleukin-8 synthesis in human lower uterine segment fibroblasts by cytokines and growth factors. Obstet Gynecol 2000; **95**:584–8.
13 Sennstrom MB, Ekman G, Westergren-Thorsson G *et al.* Human cervical ripening, an inflammatory process mediated by cytokines. Mol Hum Reprod 2000; **6**:375–8.
14 Young A, Thomson JT, Ledingham MA, Jordan F, Greer IA, Norman JE. Immunolocalization of proinflammatory cytokines in myometrium, cervix, and fetal membranes during human parturition at term. Biol Reprod 2002; **66**:445–9.
15 El-Maradny E, Kanayama N, Halim A, Maehara K, Sumimoto K, Terao T. The effect of interleukin-1 in rabbit cervical ripening. Eur J Obstet Gynecol Reprod Biol 1995; **60**:75–80.
16 Elliott CL, Loudon JA, Brown N, Slater DM, Bennett PR, Sullivan MH. IL-1beta and IL-8 in human fetal membranes: changes with gestational age, labor, and culture conditions. Am J Reprod Immunol 2001; **46**:260–7.
17 Ekman G, Granstrom L, Malmstrom A, Sennstrom M, Svensson J. Cervical fetal fibronectin correlates to cervical ripening. Acta Obstet Gynecol Scand 1995; **74**:698–701.
18 Bishop EH. Pelvic scoring for elective induction. Obstet Gynecol 1964; **24**:266–8.
19 Stewart JA, Bulmer JN, Murdoch AP. Endometrial leucocytes: expression of steroid hormone receptors. J Clin Pathol 1998; **51**:121–6.
20 Kemp B, Winkler M, Maas A *et al.* Cytokine concentrations in the amniotic fluid during parturition at term: correlation to lower uterine segment values and to labour. Acta Obstet Gynecol Scand 2002; **81**:938–42.
21 Hebisch G, Grauaug AA, Neumaier-Wagner PM, Stallmach T, Huch A, Huch R. The relationship between cervical ripening, interleukin-6 and interleukin-8 during term labour. Acta Obstet Gynecol Scand 2001; **80**:840–8.
22 Rozenberg P, Goffinet F, Hessabi M. Comparison of the Bishop score, ultrasonographically measured cervical length, and fetal fibronectin assay in predicting time until delivery and type of delivery at term. Am J Obstet Gynecol 2000; **182**:108–13.
23 Kelly AJ, Kavanagh J, Thomas J. Relaxin for cervical ripening and induction of labour. Cochrane Database Syst Rev 2001; **2**:CD003103.
24 Vaisanen-Tommiska M, Nuutila M, Ylikorkala O. Cervical nitric oxide release in women preterm. Obstet Gynecol 2004; **103**:657–62.

25 Ekman-Ordeberg G, Stjernholm Y, Wang H, Stygar D, Sahlin L. Endocrine regulation of cervical ripening in humans – potential roles for gonadal steroids and insulin-like growth factor-1. Steroids 2003; **68**:837–47.
26 Lysell J, Stjernholm Vladic Y, Ciarlo N, Holmgren A, Sahlin L. Immunohistochemical determination of thioredoxin and glutaredoxin distribution in the human cervix, and possible relation to cervical ripening. Gynecol Endocrinol 2003; **17**:303–10.
27 Sennstrom MB, Brauner A, Bystrom B, Malmstrom A, Ekman G. Matrix metalloproteinase-8 correlates with the cervical ripening process in humans. Acta Obstet Gynecol Scand 2003; **82**:904–11.
28 Sugano T, Narahara H, Nasu K, Arima K, Fujisawa K, Miyakawa I. Effects of platelet-activating factor on cytokine production by human uterine cervical fibroblasts. Mol Hum Reprod 2002; **7**:475–81.
29 Yoshida M, Sagawa N, Itoh H *et al.* Prostaglandin $F_{2\alpha}$, cytokines and cyclic mechanical stretch augment matrix metalloproteinase-1 secretion from cultured human uterine cervical fibroblast cells. Mol Hum Reprod 2002; **8**:681–7.
30 Stjernholm Y, Sahlin L, Akerberg S *et al.* Cervical ripening in humans. potential roles of estrogen, progesterone, and insulin-like growth factor-1. Am J Obstet Gynecol 1996; **174**:1065–71.
31 Stjernholm Y, Sahlin L, Malmstrom A, Barchan K, Eriksson HA, Ekman G. Potential roles for gonadal steroids and insulin-like growth factor 1 during final cervical ripening. Obstet Gynecol 1997; **90**:375–80.
32 Bokstrom H, Brannstrom M, Alexandersson M, Norstrom A. Leucocyte subpopulations in the human uterine cervical stroma at early and term pregnancy. Hum Reprod 1997; **12**:586–90.
33 Thomson AJ, Telfer JF, Young A *et al.* Leucocytes infiltrate the myometrium during human parturition: further evidence that labour is an inflammatory process. Hum Reprod 1999; **14**:229–36.
34 Winkler M, Fischer DC, Ruck P *et al.* Parturition at term. parallel increases in interleukin-8 and proteinase concentrations and neutrophil count in the lower uterine segment. Hum Reprod 1999; **14**:1096–100.
35 Osman I, Young A, Ledingham M *et al.* Leukocyte density and proinflammatory cytokines expression in human fetal membranes, decidua, cervix and myometrium before and during labour at term. Mol Hum Reprod 2003; **9**:41–5.
36 Schmitz T, Leroy MJ, Dallot E, Breuiller-Fouche M, Ferre F, Cabrol D. Interleukin-1 beta induces glycosaminoglycan synthesis via the prostaglandin E(2) pathway in cultured human cervical fibroblasts. Mol Hum Reprod 2003; **9**:1–8.